

**CALDON BIOTECH INC.**  
**Thyroid Stimulating  
 Hormone (TSH) ELISA**

Catalog No. TS045S  
 (96 tests)

**INTENDED USE**

The CBI TSH ELISA kit is used for the quantitative measurement of TSH in human serum or plasma.

**CLINICAL UTILITY**

Thyroid Stimulating Hormone (TSH) is a glycoprotein hormone secreted by the pituitary gland and regulates the synthesis/ release of T3 and T4 by thyroid gland. TSH has two subunits, namely alpha and beta. The alpha subunit is similar to the alpha subunit found in LH, FSH and hCG glycoprotein hormones. However, the beta subunit is specific and differs from hormone to hormone. The serum TSH measurement is one of the most important tools in the diagnosis of thyroid disorders. Increased serum TSH is an early and sensitive indicator of decreased thyroid reserve and overt primary hypothyroidism. Decreased of TSH levels is an indicator of TSH-independent hyperthyroidism (Graves disease). The sensitivity of this ELISA test is 0.05 $\mu$ U/mL.

**PRINCIPLE OF THE TEST**

The CBI TSH is a solid phase sandwich ELISA method. The samples, and anti-TSH-HRP conjugate are added to the wells coated with Mab to TSH beta subunit. TSH in the patient's serum binds to anti-TSH MAb on the well and the anti-TSH second antibody then binds to TSH. Unbound protein and HRP conjugate are washed off by wash buffer. Upon the addition of the substrate, the intensity of color is proportional to the concentration of TSH in the samples. A standard curve is prepared relating color intensity to the concentration of the TSH.

**MATERIALS PROVIDED**

1. Microwells coated with TSH MAb (12x8x1 wells). Total 96 wells.
2. TSH Standard: 7 vials (0.7 mL each). Ready to use.
3. Enzyme Conjugate: 1 bottle (12 mL). Ready to use.
4. TMB Substrate: 1 bottle (12 mL). Ready to use.
5. Stop Solution: 1 bottle (8 mL). Ready to use.

6. Wash Concentrate: 1 bottle (50 mL/10X).

**STORAGE AND STABILITY**

1. Store the kit at 2 - 8°C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.

**WARNINGS AND PRECAUTIONS**

1. Potential biohazardous materials: The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984
2. This test kit is designed for in vitro diagnostic use only.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. It is recommended that standards, control and serum samples be run in duplicate.
6. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

**SPECIMEN COLLECTION HANDLING**

1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at (2-8°C) for 5 days. If storage time exceeds 5 days, store frozen at (-20°C) for up to one month. 3. Avoid multiple freeze-thaw cycles.
3. Prior to assay, frozen sera should be completely thawed and mixed well.
4. Do not use grossly lipemic specimens.

**REAGENTS PREPARATION**

10X Wash Buffer Concentrate: To prepare working wash buffer, add the contents of the bottle to 450 ml of distilled water. Store at room temperature.

Classification	Normal Range (µIU/mL)
Adults	0.4-4.2
Newborn (1-4 days)	1.0-39
2-20 weeks	1.7-9.0
21 weeks-20 years	0.7-6.4

**ASSAY PROCEDURE**

Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder
2. Pipet 50 µL of TSH standards, control and patients.
3. Add 100 µL of ready to use enzyme conjugate to all wells.
4. Cover the plate and incubate for 60 minutes at room temperature (18- 26°C).
5. Remove liquid from all wells. Fill wells with working wash buffer. Wash three times. Blot on absorbent paper towels.
6. Add 100 µL of TMB substrate to all wells.
7. Incubate for 15 minutes at room temperature.
8. Add 50 µL of stop solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 20 minutes after adding the stopping solution.

**LIMITATIONS OF THE TEST**

1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
2. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

**PERFORMANCE CHARACTERISTICS**

**1. Correlation with a Reference ELISA kit:**

A total of 110 sera were tested by this ELISA and a reference ELISA kit. Results were as follows:

Correlation	Slope	Intercept
0.91	0.9	0.37

**CALCULATION OF RESULTS**

The standard curve is constructed as follows:

1. Check TSH standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached.
2. To construct the standard curve, plot the absorbance for the TSH standards (vertical axis) versus the TSH standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

**2. Precision  
Intra-Assay**

Serum	No. of Replicates	Mean (µIU/mL)	Standard Deviation	Coefficient of Variation%
Normal	16	24.0	1.36	5.67
Low	16	9.70	0.83	8.56
High	16	0.33	0.03	8.98

**EXPECTED VALUES**

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values for TSH may be used as initial guideline ranges only:

**Inter-assay**

Serum	No. of Replicates	Mean (µIU/mL)	Standard Deviation	Coefficient of Variation%
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Normal	10	25.9	1.76	6.80
Low	10	10.2	0.83	8.14
High	10	0.47	0.04	8.51

**1. Sensitivity**

The sensitivity was determined by calculating the mean plus 2SD of the standard zero point tested 20 times in the same run.]

Serum	No. of Replicates	Mean (µIU/mL)	Standard Deviation	Mean + 2SD (Sensitivity)
Zero Standard	20	0.01	0.02	0. (µIU/mL)

**2. Recovery**

Known quantities of TSH were added to a serum that contained a low concentration of TSH.

Expected Value(µIU/mL)	Recovered (µIU/mL)	Percentage of Recovery
17.70	17.04	96.3
13.60	12.40	91.0
04.54	4.45	98.0

**3. Linearity**

Two different patient samples were diluted with the "0" calibrator to 1:2, 1:4 and 1:8. TSH values were assayed and results were corrected with the dilution factor. The results of these dilution tests are as follows:

Serum	Original Value (µIU/mL)	Percentage of Recovery		
		1:2	1:4	1:8
		93.3	98.6	84.8
		109.0	89.4	85.0

**REFERENCES:**

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